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E2F transcription regulation: an orphan cyclin enters the stage

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Marked cyclin protein oscillations over the cell cycle ensure tight regulation of all cell cycle transitions. Despite expression patterns closely mirroring those of cyclin A, cyclin F has long been regarded as an odd outlier within the cyclin family. Constituting part of an E3 ubiquitin ligase, its main role was seen as highly restricted to timely degradation of very few key substrates to ensure termination of one error-free round of replication. Now, a recent series of studies suggests that cyclin F has very similar roles as its closest relatives, merely mediated through a very different mechanism.

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See also: **R Yuan *et al*** and **K Burdova *et al*** (October 2019)

The meticulous execution of the cell division cycle is a fundamental premise for life. Fine-tuned feedback mechanisms and checkpoints ensure multiple layers of control throughout the cell cycle. There is precise regulation at all levels including transcription, post-translational modifications (PTM), and through tightly regulated protein degradation. At the post-translational level, cyclin-dependent kinase (CDK) complexes are crucial for timely progression through the cell cycle phases, particularly enabling passages that connect the different stages. CDKs are activated by a cyclin subunit, which often vastly increase the efficiency of CDK substrate phosphorylation through binding to a Cymotif, defined by the amino acid motif RXL, on their target proteins.

A notably different member of the cyclin family is cyclin F, which does not form a complex with a CDK. Instead, cyclin F mainly controls the stability of other proteins by serving as targeting subunit of a Cullin 1-SKP1-RING family ubiquitin ligase—whose "F-box protein" subunits in fact owe their name to it. Cyclin F accumulates in the late S and G2 phases, where it mediates the degradation of a distinct repertoire of key proteins. Cyclin F so far has been known for safeguarding the orderly termination of a single round of replication by ensuring timely degradation of CDC6, CP110, and NUSAP (Galper *et al*, 2017). Accordingly, failure to suppress CDC6 protein levels sensitizes cells to commencing a new round of replication uncoupled from mitosis (Walter *et al*, 2016). Cyclin F activity can also affect genome maintenance via its degradation of RRM2, SLBP, and EXO1 (Galper *et al*, 2017). Intriguingly, a subset of cyclin F interactors are not degraded, but cyclin F rather competes with distinct cyclin/CDK complexes for their Cy motif binding sites. This *modus operandi* has been shown for inactivation of the transcription factor B-Myb, where cyclin F suppresses cyclin A-CDK-mediated B-Myb activation (Klein *et al*, 2015). Cyclin F thereby guards against premature entry into mitosis, through restraining B-Myb activity.

At the transcriptional level, the E2F family of transcription factors is key for timely progression through the cell cycle. E2F transcription factors control the expression of genes involved in replication initiation and in G1/S and G2/M transitions, among many other targets. The E2F family comprises three activators (E2F1, E2F2, and E2F3a), four repressors (E2F3b, E2F4, E2F5, and E2F6), and two atypical repressors (E2F7 and E2F8). Within the family of E2F transcription factors, we find a self-regulating feedback loop in which activators bind to the promoters of repressors and *vice versa*. Interestingly, the consensus DNA motif TTSSCGCC (S = C or G) is recognized by both E2F-family activators and repressors (Westendorp *et al*, 2012), which further counterbalances the levels of transcription at sites common to both activators and repressors (Thurlings & de Bruin, 2016). Of note, TTSSCGCC motif-containing genes responsible for mitotic entry (CCNA2, CDC2, CCNB1, etc.) are not under the control of the atypical repressors E2F7/8 (Westendorp *et al*, 2012). Instead, many of these genes are co-regulated via the transcription factor B-Myb.

Cyclin F was previously implicated in transcriptional regulation mainly by control of the phosphorylation status of B-Myb (Klein *et al*, 2015). A series of recent investigations, including two studies in *The EMBO Journal*, has now unraveled extensive links between cyclin F activity and transcriptional regulation through degradation of the transcriptional activators E2F1, E2F2, and E2F3a (Burdova *et al*, 2019; Clijsters *et al*, 2019), as well as the atypical repressor E2F7 (Yuan *et al*, 2019). Thereby, cyclin F emerges as a key factor in ensuring the orderly initiation, execution, and termination of precisely one round of replication at several levels. Furthermore, it is clear that unscheduled E2F activity resulting from absence of cyclin F leads to a marked disturbance in cell cycle progression and increase in DNA damage (Clijsters *et al*, 2019; Yuan *et al*, 2019).

The timely degradation of E2Fs via cyclin F helps to finally answer the long-standing question on how the activity of E2F activators, after peaking in G1 and S phases, drastically declines once cells progress through G2 (Clijsters *et al*, 2019) (Fig 1A). The

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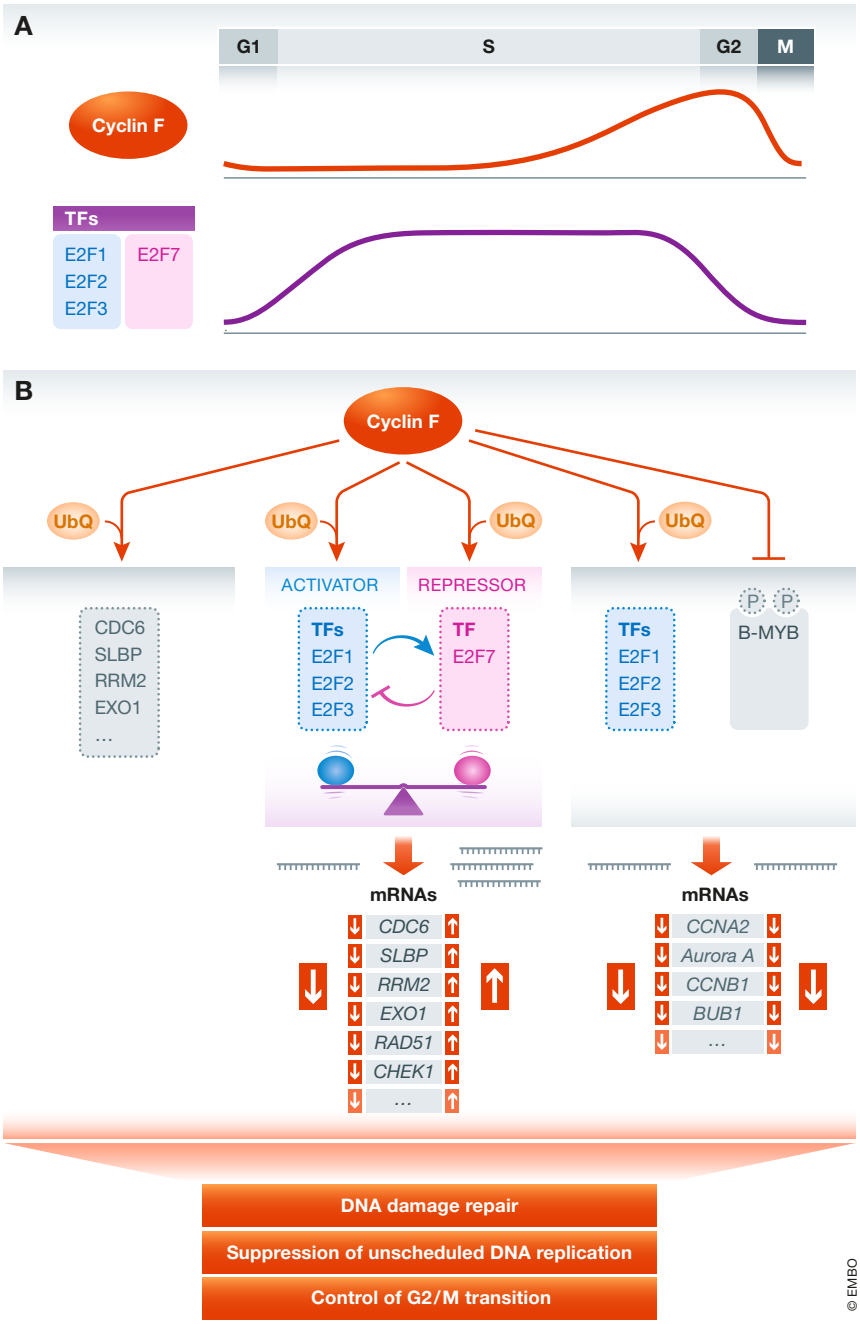

Figure 1. Cyclin F acts at three levels to promote cell cycle regulation.

(A) Diagram depicting the protein levels of cyclin F throughout the cell cycle and the dependencies of the protein levels of E2F1, E2F2, E2F3a, and E2F7 on the activity of cyclin F. (B) Originally, cyclin F was thought to only directly act on a subset of substrates via its E3 ligase activity (tier 1). Cyclin F activity regulates transcriptional control of target genes involved in replication and DNA damage repair (tiers 2+3) by controlling the levels of the transcription activators E2F1, E2F2, and E2F3a and the atypical transcription repressor E2F7.

importance of cyclin F in regulating E2Fs is further unraveled in the study by Yuan *et al* (2019), which shows that cyclin F is also responsible for timely degradation of the atypical repressor E2F7. Given the overlap of DNA binding sites, the finding that two types of E2Fs, activators and the atypical repressor E2F7, are under the control of the same E3 ligase seems surprising at first sight. In the future, it will be of great interest to decipher how exactly this delicate balance can lead to the timely activation of certain genes while it causes inactivation of others. Conceivably, yet another level of fine-tuning may be achieved by controlling the relative protein levels of different E2F transcription factors.

Interestingly, there is a remarkable overlap between the targets of E2F transcriptional control and the targets of regulated degradation by cyclin F, as observed e.g. for CDC6, EXO1, RRM2, or SLPB. This directly implies that regulation of cyclin F substrates can happen at three different levels: post-translationally, via controlled proteasomal degradation, and indirectly via the degradation of selected E2F family members (see Fig 1B). With the additional loss of control over timely degradation of E2F transcription factors, the absence of cyclin F results in over-licensing and mitotic transcription (Clijsters *et al*, 2019). In addition, cyclin F has now also been shown to regulate DNA damage response genes responsible for base excision repair, nucleotide excision repair, DNA mismatch repair, and homologous recombination, which were previously weakly linked to cyclin F on the substrate level (Yuan *et al*, 2019). With a simultaneous deficiency in the repair machinery, dysfunction of cyclin F will inevitably lead to replication stress.

The study by Burdova *et al* (2019) highlights the direct consequences of an imbalance within this E2F feedback system. Burdova *et al* further uncover how loss of cyclin F is directly connected to a synthetic lethal interaction with the ATR-CHK1 pathway. This pathway is crucially important in suppressing DNA replication stress, which is elevated when cyclin F is suppressed. The synthetic lethality indeed opens new perspectives for cancer therapy and for sensitizing tumorigenic cells for chemotherapy, as suggested by Burdova *et al* (2019). Indeed, ubiquitin ligases are emerging as promising targets for cancer therapy. This in part owes to the notion that inhibition of ubiquitin ligases may display reduced side effects due to a more narrow range of direct enzymatic targets than is often observed for kinases. In addition, ubiquitin ligases also bear the potential to be used in personalized treatment, since predisposing weaknesses could directly be evaluated via distinct sets of accumulating target biomarkers. Therefore, this synthetic lethal interaction has the potential to be exploited as future treatment

option. Currently, CHK1 inhibitors as anticancer treatments are being developed and advanced toward the clinic; hence, CCNF status can be taken into account as a relevant biomarker. Several cancers display elevated levels of cyclin F, which counteracts the efficiency of CHK1 treatment. Alternatively, for cyclin F-overexpressing tumors, the development of cyclin F inhibitors to force uncontrolled dividing cells into lethal levels of replication stress appears sensible.

Taken together, it has become apparent that cyclin F is not a niche factor in the control of the cell cycle. On the one hand, cyclin F acts in the short term (in concert with other CDK inhibitors), targeting RXL-containing proteins via their retention in a hypo-phosphorylated state. Expanding from here, cyclin F also targets a subset of these proteins for proteasomal degradation to generate a more permanent off-state. In its emerging new role, cyclin F indirectly also shuts down transcription of these very same target genes. Thus, there is an emerging role of cyclin F acting broadly and within all three layers of control on the cell cycle, a fast, PTM-mediated response, a long-term response via regulated degradation, and finally, via the indirect control of transcription factors at mRNA levels.

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